PYTHIUM RESISTANCE IN ALFALFA

by

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INTRODUCTION

Pythium species, world wide in distribution, occur in soil and water as saprophytes on decaying plants and animals and as parasites on the subterranean portion of many plants, being one of the most common incitants of root rots and damping-off. Pythium damage in alfalfa appears to be confined to seedlings (27,28,44) in the form of damping-off.

Pathogenic damping-off (3) has been categorized as seed rot and pre- and postemergence damping-off. Seed rot is the result of invasion by the pathogen during the early stages of germination, killing the seed before it can sprout. Pre-emergence damping-off is caused by infection whereby seedlings are killed after the seed rotting phase but prior to emergence. Postemergence damping-off is first characterized by the pinched and brownish appearing hypocotyls near the soil surface and subsequently by fallen plants (Plate I).

Failure to obtain satisfactory stands of seedling alfalfa is often credited to low quality seed, consequently it is difficult to estimate the actual damage resulting from damping-off.

Several approaches to the control of Pythium have been suggested. The application of lime (5,7,8) to acid soils, in addition to its other merits, tends also to lessen damping-off. Deep plowing in some cases has reduced damping-off as Pythium appears more abundant in the surface soil than at depths below four inches. Date of planting (3,7,8) can also be rather effective because of the influence of climatic factors on the host

EXPLANATION OF PLATE I

Eleven-day-old Cody alfalfa seedlings illustrating various symptoms of *Pythium* infection. From left: a rotted seed, two seedlings with girdled hypocotyls typical of post-emergence damping-off, a seedling showing the characteristic small sunken brownish lesions found on the hypocotyl and cotyledons, three seedlings exhibiting adventitious roots which have formed above the area of damping-off allowing the plants to continue growth. Seedling at right was grown in uninfested sand.

PLATE I

plant.

Crop rotation appears inadequate to control the disease because *Pythium* species most pathogenic to alfalfa also attack most other commonly grown field crops. These organisms can also exist in the soil indefinitely as saprophytes without loss of virulence, and there is evidence (26) of increased pathogenecity in soil being fallowed under certain conditions.

Fungicidal seed treatments (1,7,17,23,31,41,51,54) have given varying degrees of control, but the results have not been spectacular. Much improvement in this area is no doubt forthcoming as a result of increased research.

Treating seeds with gibberellic acid (24,59) prior to and at the time of planting was investigated and found to increase damping-off significantly. Antibiotics (27,57) have produced variable results and are not of practical value at the present time.

The development and use of resistant varieties appears to be the preferred economic solution. The present research was initiated to explore this possibility, the objectives being:

1. To develop a reliable and usable method of testing for resistance.

2. To determine factors affecting resistance.

3. To determine sources of resistance.

4. To determine if resistance might be increased by selection.

5. To study the relative reaction of alfalfa varieties and selections to different *Pythium* species.

REVIEW OF LITERATURE

Distribution, Host Range, and Relative Pathogenecity

Middleton (45) listed over 220 plant species having known susceptibility to *Pythium* species.

Buchholtz and Meredith (10) found *Pythium debaryanum* Hesse to be the primary incitant of damping-off of alfalfa seedlings in Iowa in 1934, 1936, and 1937. McDonald (44) identified all 447 isolates from damped-off alfalfa seedlings in Manitoba as *P. debaryanum*. Gregory et al. (27) found *P. debaryanum* and *P. ultimum* Trow the most pathogenic on alfalfa of five *Pythium* species studied. Halpin et al. tested the relative pathogenecity of seven *Pythium* species on red clover (29) and five on alfalfa, Ladino white clover, red clover, and sweetclover (30). They reported *P. debaryanum*, *P. irregulare* Buis., and *P. ultimum* the most pathogenic and noted that alfalfa and sweetclover were the most susceptible.

Kilpatrick et al. (40) reported that *P. debaryanum* caused the most severe pre-emergence killing of red clover seedlings of 28 fungal isolates studied at Wisconsin. Isolations by Jackobs (38) from rotting sweetclover seeds in Wisconsin soils were predominantly pythiaceous. *P. debaryanum* and *P. ultimum* were most pathogenic.

Richter (48) found the *Pythium* species, especially *P. debaryanum*, to be major incitants of root rot of lupines in Germany.

Seedling diseases were considered the most important dis-

eases of sugar beets in Michigan by Coons and Stewart (11) and P. debaryanum was the most commonly isolated incitant. Buchholtz (7) reported that P. debaryanum caused over 95 percent of the damage to germinating and emerging sugar beet seedlings in northern Iowa.

Arndt (2) reported that P. ultimum was a seedling parasite of cotton. Thomas (55) isolated P. ultimum from rotted castorbean seeds at Beltsville, Maryland, more frecuently than all other fungi combined. Pythium irregulare was one of the most destructive of the fungi causing damping-off of red pine (Pinus resinosa Ait) seedlings in sandy Wisconsin soils (50).

Curme (13) found the Pythium species, particularly P. debaryanum, to be the most common isolate from soil incubated corn seeds at Manhattan, Kansas. Hoppe and Middleton (37) found P. debaryanum and P. irregulare the most prevalent isolates from diseased corn seedlings in several Wisconsin soils. Hoppe (35) reported that Pythium species were the main incitants of seed decay and seedling disease of corn.

Pythium debaryanum was the most frequent and most pathogenic organism isolated from diseased oats from widely separated parts of Iowa by Welch (58), but P. irregulare was more common in some light sandy soils.

Edgerton (18) considered the Pythium species the most important ones attacking sugarcane roots. Pythium species were by far the most prevalent fungi obtained from root and crown tissues of smooth brome grown in Wisconsin and South Dakota soils (56). Buchholtz (9) concluded that P. debaryanum was the principal

cause of seed rotting of crested wheatgrass in South Dakota.

Factors Affecting Pathogenecity

Moisture. Beach (3), working with several vegetable crops, found that the severity of attack by *Pythium ultimum* increased with a rising soil moisture content up to the point of saturation. Beach and Chen (4) noted that tomato seedlings were highly susceptible to *Pythium ultimum* for about three days after transplanting and that thorough drying of the infested soil largely prevented damping-off as the seedlings passed this susceptible period.

Kreitlow et al. (41) and Graham et al. (25), investigating some grass and legume forage crops, also reported that damping-off increased as the soil moisture content increased. The former noted that *Pythium* remained virulent for long periods in moist soil but lost its potency rapidly in dry soil.

Grandfield et al. (26) concluded that *Pythium*-incited damping-off of young alfalfa stands became more severe with increased fallow periods prior to seeding because the accumulated soil moisture favored the development of the *Pythium* species.

Coons and Stewart (11) and Buchholtz (7) found that the greatest sugar beet losses due to damping-off occurred on soils having poor natural drainage.

Pythium-incited damping-off of pine seedlings increased from a minimum at 13 percent to a maximum at 100 percent soil moisture content (49). Air humidity had no influence on the severity of infection.

Hoppe (36) using muck soil previously air-dried for 6 years clearly showed the necessity of water for Pythium species to cause seedling blight of corn. A wetting period of about 5 days was required for the first evidence of Pythium activity, and 10 to 15 days of wetting was required for the fungus to regain its full pathogenic potential. Flor (21) noted that Pythium damage to corn increased as the soil water content increased, being especially noticeable when the soil contained more than 50 percent of its moisture holding capacity. In wet soils, however, damage increased as temperature increased.

Temperature. Leach (42) and Beach (3) investigated the pathogenecity of P. ultimum and other soil-borne pathogens with several low- and high-temperature garden crops over a wide range of temperatures. In all combinations of host and pathogen, preemergence Pythium infection was most severe at temperatures relatively less favorable to the host than to the pathogen as measured by the ratio of their growth rates.

The optimum temperature for most rapid mycelial growth on artificial media by some of the more pathogenic Pythium species was 26 to 30°C. (7,21,25,29,45,48,49,58). The minimum temperature at which growth occurred was 1 to 4°C. (45) and the maximum 36 to 40°C. (21,45). Middleton (45) found that the climatic source of a Pythium isolate did not determine its ability to develop at a given temperature, as all isolates of the same species gave the same temperature reactions regardless of origin.

Roth and Riker (49) found no relationship between Pythium growth rate in culture and the amount of damping-off of pine

seedlings at several temperatures.

Factors Affecting Resistance

Temperature. Dickson (14) and Dickson and Holbert (15) determined the temperature influence upon the development of seedling blight of corn and wheat as primarily a host response.

Studies with oat seedlings (58) revealed that although Pythium infection remained high at the higher temperatures it had no serious effect on the growth and development of the plants because they were able to produce new roots faster than the old ones were destroyed.

Investigators studying seed and seedling resistance to the Pythium species, in general, found that high-temperature crops were more resistant at high temperatures and low-temperature crops were more resistant at lower temperatures. Crops exhibiting greater resistance as the temperature increased included corn (5,33,34,39,51), common lespedeza (25), cotton (2), red pine (49), oats (58), sugarcane (21), and watermelon (42). Crops expressing increased resistance at lower temperatures were alfalfa (5,6,30, 31), alsike clover (5), Ladino white clover (5,25,30), sweetclover (30,31) and sugar beet (7).

Hanson (31), testing some common legumes, including Ranger alfalfa, in soil naturally infested with damping-off organisms at 16,20,24, and 28°C., obtained the best stands at 16°C. Buchholtz (5,6) found less damping-off of alfalfa seedlings in acid soil at 9°C. than at 20 to 25°C.

pH. Buchholtz (6) found damping-off to be associated with

acidity in Iowa soils. Alfalfa seedlings grown on three acid soils were 16-49 percent diseased while on neutral soils only 6-7 percent were diseased.

Buchholtz (7) also reported that P. debaryanum caused especially poor stands of sugar beets in soils below pH 6.5. A 1933 survey of sugar beet fields in northern Iowa revealed a correlation of 0.745 between soil pH and percentage stand. Liming increased seedling emergence but the improvement was not sufficient to warrant its expense.

Roth and Riker (49) found that growth of P. irregulare occurred on potato-dextrose agar from pH 3.7 to near 9, with maximum growth occurring between pH 5 and 8. Optimum development of red pine seedlings occurred between pH 4.7 and 6. A close association was found between the rate of mycelial growth of Pythium in culture and the severity of damping-off of pine seedlings below pH 6.8. Above pH 6.8 mycelial growth declined while damping-off continued to increase. The authors associated this increased susceptibility with retarded host development found under alkaline conditions.

Age. Halpin and Hanson (28) checked the effect of age on resistance of alfalfa, sweetclover, red clover, and Ladino white clover seedlings to five Pythium species. They found that when infestation was delayed until one day after seeding no seed rotting was observed, but pre-emergence killing occurred. Two-day-old red clover and Ladino white clover plants were immune to Pythium attack, while alfalfa and sweetclover seedlings were not immune until the third day after planting. Resistance to the less

pathogenic isolates was acquired faster than to the more pathogenic ones.

Gregory et al. (27) found that resistance of alfalfa seedlings to damping-off incited by five Pythium species increased with age until there was little or no kill when inoculations were made 14 or more days after seeding.

McDonald (44), studying pathogens from diseased roots of alfalfa plants 1 month and 1, 2, and 3 years of age, isolated P. debaryanum from 17 percent of the 1-month-old plants but from none of the older plants.

Buchholtz (6) noted that alfalfa seedlings were especially susceptible to Pythium infection during the germination and emergence stages and became more resistant with age, but they were not immune to local lesions on the hypocotyls and primary roots until fully established.

Arndt (2) concluded that P. ultimum was strictly a seedling parasite of cotton, as all plants alive 30 days after seeding were capable of rapid recovery.

Hooker and Dickson (33) found that Pythium resistance of all excised corn embryos tested increased as germination progressed but that some embryos acquired resistance earlier.

Seed Quality. Jackobs (38) concluded that maturity was the most important factor in determining the ability of sweetclover seed to germinate in the presence of Pythium.

Thomas (55) considered immaturity, usually due to early defoliation by drought, frost, chemcial desiccants, or disease, as the chief factor responsible for the predisposition of castorbean

seed to pre-emergence damping-off. Castorbean seeds harvested separately from the first, second, and third sequential sets of racemes differed markedly in stands produced in favor of the more mature seed.

Welch (58) found ungerminated seeds and seedlings from 4-year-old oat seed to be more subject to Pythium injury than those from 1-year-old seed. He attributed the increased resistance of the younger seed to more rapid germination and greater viability. This allowed the resulting seedlings to escape parasitic invasion and development.

Rush and Neal (51) reported immaturity and frost damage of corn seed at harvest time to be major factors in determining stands at low temperatures. Hoppe (35) also mentioned aged corn seed as more susceptible to attack by soil fungi. Curme's (13) data, however, showed cold tolerance to improve with age of seed.

Physical Seed Damage. Tatum and Zuber (54) found a close relationship between pericarp injury of seed corn and stand. The seriousness of the break depended upon how direct an entry it provided for pathogens to reach the embryo. They considered improper processing methods responsible for considerable unnecessary injury to commercial seed corn. They found intact pericarp more effective than fungicidal seed treatment in obtaining stands of corn.

Hoppe and Middleton (37) recorded near 100 percent Pythium-incited seed decay in wounded corn kernels planted at low temperatures in some Wisconsin soils. Rush and Neal (51), however, did not find seed coat injury to be a major factor in determining

stands of corn.

Seed Size. Welch (58) planted primary and secondary oat kernels in *P. debaryanum* infested soil to study their comparative resistance. In general, the plants grown from primary seeds were as seriously attacked as plants from the secondary seeds but tended to produce better plants. This was attributed to vigor and not to susceptibility differences between the two groups.

Ploidy. Welch (58) found wild species and cultivated varieties of oats having 21 chromosomes more resistant to *Pythium* attack than members of the 14- or 7-chromosome groups.

Maternal Influence. Jackobs (38) found differences in the severity of *Pythium*-incited rotting of seed produced by individual sweetclover plants in the field. However, all mature seeds produced in the greenhouse were resistant to seed rot. He concluded that these differences were not due to a specific interaction of the organism and a gene or genes for resistance of the host. Rather, he considered it due to seed-producing plant characters as resistance or susceptibility to certain diseases or adverse environmental conditions that influenced seed development in the environment in which the plant was growing.

Hoppe (35) and Pinnell (46) emphasized that the female parent in corn exerted the stronger influence on the ability of seedlings to resist blight. Crane (12) found evidence of resistance to *Pythium ultimum* in corn seedlings in both the maternal pericarp tissues and within the germinating embryo. The two appeared distinct and genetically independent.

By using excised corn embryos Hooker and Dickson (33) were

able to find a definite genetic pattern for Pythium resistance that had not been attained with intact kernels. They suggested the maternal influence was due to the quality and quantity of food materials provided by the endosperm to the embryo both during differentiation on the maternal plant and during the early stages of germination.

Resistance to Hyphal Penetration. Hawkins and Harvey (32) concluded that resistance to Pythium incited tuber rot by the White McCormick potato variety was due to the resistance of its unusually thick cell walls to penetration by the pathogen.

McClure and Robbins (43) determined the resistance of cucumber seedlings to infection by a Pythium-type fungus was associated with and perhaps significantly dependent upon rapid lignification of living parenchymatous cell walls in the tissue surrounding the region of infection. These cell walls did not become lignified in susceptible or noninfected resistant plants.

Dickson and Holbert (15) found that the cortical cell walls of corn strains resistant to seedling blight were abundantly impregnated with suberin, while little or no suberin was evident in these cells in susceptible strains. They found that fungal penetration was largely inhibited by cellulose cell walls reinforced with either lignin or suberin.

Indirect Tests for Resistance

Cold Test. Dickson and Holbert (16) considered "inheritable resistance" in corn to seedling blight similar in nature to "induced resistance" brought about by high temperature. This re-

lationship led to the "cold test" (13,33,46,51,53,54) of corn which Hoppe (35) declared as essentially a test against soil-borne diseases. Thomas (55) used a similar test to evaluate the relative Pythium resistance of castorbean seed lots.

Permability Tests. Turbidity tests by Tatum (53) and Curme's collodial indexes (13) indicated that the amount of diffusate leached from corn kernels soaked in water was related to their "cold test reaction". Crane (12) used a more direct measure of the diffusate, that of sugar determination. He calculated correlation coefficients of between -0.85 and -0.91 for seedling survival in muck soil and the amount of reducing sugars leached from duplicate seed samples from five lots of corn seed.

Presley (47) preferred conductivity readings of distilled water leachings from cotton seed to turbidity tests as a method of evaluating protoplast permability which he considered a predisposition to seedling disease.

MATERIALS AND METHODS

Inoculum

Sources. Culture A-21-56 of Pythium irregulare Buis. served as the source of inoculum in all studies except those involving Pythium species-host interaction. This culture was isolated in 1948 by Dr. P. C. Duffield from diseased alfalfa seedlings growing near Manhattan, Kansas, and identified by Dr. C. L. Kramer, mycologist at Kansas State University.

Other Pythium cultures used included I-1 and 58-15C of P.

debaryanum Hesse from Iowa State University and cultures W-1 of *P. ultimum* Trow and W-2 of *P. irregulare* Buis. from the University of Wisconsin.

Media. All cultures were maintained on PDA (potato-dextrose agar) slants in a refrigerator. Reisolation from diseased plants was not attempted as there was no apparent loss of virulence in the cultures during the studies.

Two percent PDA served as the major source of medium. This was prepared by mixing 20 g. each of agar and dextrose into water in which 200 g. of chopped potatoes had been autoclaved 30 minutes at 15 psi and diluting with water to 1,000 ml. PDB (potato-dextrose broth) was prepared in the same manner except agar was not included. Distilled water was used in the preparation of all inoculum.

Increase. Inoculum was increased by transferring small amounts of mycelium from slants to the center of Petri dishes containing approximately 20 ml. of PDA and incubating at room temperature for 3 days.

Increasing inoculum in PDB was accomplished by transferring mycelium from slants into 1,000 ml. Erlemeyer flasks containing 500 ml. of the sterile medium. Flasks containing inoculum were kept on a shaker for 7 days. Shaking was later discontinued as it was found unnecessary. This greatly decreased losses due to contamination.

Preparation. The adopted method of preparing inoculum on PDA consisted of placing the contents of 2 Petri dishes containing 3-day-old cultures into a Waring blender with 200 ml. of dis-

tilled water and blending for 20 seconds. The contents were then poured into a tea strainer having openings 3/4 mm. in diameter. That portion remaining in the strainer was added to the following aliquot.

To compensate for varying amounts of inoculum within different Petri dishes, all inoculum was bulked and thoroughly mixed after the blending process.

The contents of 2-2½ Petri dishes, depending upon the planting method used and the estimated resistance of the material being tested, diluted to 500 ml. with distilled water provided the inoculum for a flat. Control flats received sterile PDA prepared by the described method.

The mycelium from each flask of PDB was thoroughly washed in distilled water and blended in a Waring blender with 200 ml. of distilled water for 10 seconds. The average mycelium suspension from 1 flask diluted to 500 ml. served as inoculum for 1 flat.

Adopted Testing Procedures

As a result of the constant search for means of improving testing methods obviously all tests were not carried out in identical manner. To explain in detail each of the variations in this section and associate each with its respective test would be confusing to the reader. Therefore, this section is devoted to the description of the testing procedures considered by the author to be the most satisfactory of those tried, and variations from these will be explained at the appropriate times.

Sterile, moist "fine" mason's sand served as plant medium.

It was sterilized by autoclaving in 5-gallon garbage cans at 15 psi for 4 hours. Metal flats were used and sterilized by autoclaving for 30 minutes. Hands and planting equipment were washed with 95 percent alcohol to prevent contamination in the control flats. Seeds were not surface sterilized since seed borne contamination was never apparent in the control flats.

The temperature was maintained automatically in the greenhouse to within ± 2°F. of the 70°F. thermostat setting. Occasional divergences were noted on warm days. Tests were not attempted during the warmer months except in temperature controlled growth chambers.

The growth chambers utilized were designed and constructed by Dr. W. H. Sill Jr., Associate Professor of Botany and Plant Pathology and the Calvert Electric Co. of Manhattan, Kansas. Temperature was automatically maintained at ± 2°F. of the designated temperature. An incandescent and florescent lighting combination with a maximum illumination of 500 foot candles was available.

All sand used in an experiment was thoroughly mixed by hand to facilitate a uniform distribution of moisture. The flats were filled with sand and stroked with a leveling board notched on the ends to level the sand at a depth of 1 inch below the top of the flat (Plate II, Fig. 1).

Five hundred ml. of inoculum were sprinkled on each flat as evenly as possible with a plastic clothes sprinkler (Plate II, Fig. 2). A more uniform distribution of inoculum was obtained by shaking than by squeezing the bottle.

Immediately after infestation the flats were covered with two thicknesses of heavy wrapping paper to prevent rapid drying of the sand and to reduce aerial contamination.

Planting was delayed until the day following infestation to allow for a more uniform distribution of inoculum. It was assumed that the mycelial growth from the many small pieces of PDA would form an interwoven mat of fungal growth throughout the upper portion of the sand during this period.

Each flat contained 18 rows 1 inch apart. A row consisted of 25 seeds planted ½ inch deep at ½-inch intervals with the aid of a planting board (Plate III, Fig. 1). The 10 by 12 inch planting board was constructed by forcing 8-penny box nails through previously drilled slightly smaller holes until they protruded ½ inch below the lower edge of the board. The two end rows were used as borders leaving space for 16 entries. Rows were identified by marking numbers on the upper edge of flat with a black weatherproof pencil near the corresponding row in accordance with a previously prepared randomized planting plan. The randomized complete block design (20) including from four to eight replications was used. Experiments were designed to include all entries of a replication within one flat. All seeds were mechanically scarified and those plump, mature, and unbroken were planted.

Seeds for each row were counted prior to planting and placed in 2¼ by 3½ inch open-end envelopes. Planting was accomplished by dropping a seed into each nail hole. The rapid placement of 1 seed per hole was accomplished by creasing the cover of the enve-

lope lengthwise to restrict the path of seeds to a width of 1 seed and tapping on the envelope with forefinger lightly to regulate seed movement (Plate III, Fig. 1).

To facilitate uniform coverage of seeds, a press board consisting of a 3/16 by 1/4 by 12 inch wooden strip secured to the bottom side of a 1 by 1 1/2 by 12 inch board was utilized. The strip was centered 1 inch from one edge of the board so that by aligning this edge of the board over a row and pushing downward, the adjacent row was pressed shut. Sterile sand was then placed on the flats and leveled to its original depth with the leveling board (Plate III, Fig. 2).

An earlier method of planting differed in that 50 seeds were equally spaced in rows 5 inches long and ½ inch deep. The rows were approximately 2 inches apart. Seeds were covered by pressing sand in from each side of the rows and the sand leveled in the previously described manner. This method also allowed for 16 entries (Plate IV). Less inoculum was required with the latter method for the same amount of kill as the disease tended to spread from plant to plant within rows at the higher seeding rate.

Immediately following planting, the flats were again covered with wrapping paper until there was evidence of seedling emergence. Under average greenhouse conditions emergence occurred about the third day after planting. It was necessary to irrigate prior to emergence only during periods of high temperatures.

The sand was kept in a moist condition for approximately 4 days after seedling emergence by sprinkling lightly with tap

EXPLANATION OF PLATE II

Fig. 1. Sand leveled 1 inch below top of flat with leveling board.

Fig. 2. The application of inoculum with a plastic sprinkler.

PLATE II

Fig. 1

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. The planting board at left was used to make holes into which individual alfalfa seeds were placed as illustrated.

Fig. 2. Illustrating the use of the press board at right to uniformily cover seeds. Sterile sand was added and leveled to the original depth with the leveling board.

PLATE III

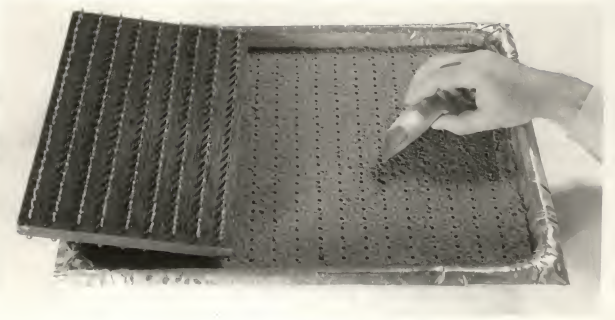

Fig. 1

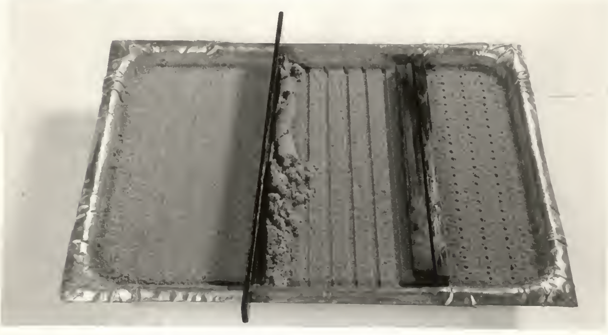

Fig. 2

water.

Notes were taken on the apparent differences in rates of emergence and vigor exhibited among entries. A count of all emerged plants was recorded between 6 and 8 days after planting, except the period was extended when smaller seeds were included.

Within a day or so after the emergence count was taken, water was withheld in order to make conditions unfavorable for the recovery of the heavily infected plants. Plants were not watered until there was an apparent need for water by the healthier plants at which time the sand was saturated. This process was repeated as necessary to eliminate weak plants.

Final counts, which generally included all living plants, were made between 2 and 3 weeks after the planting date. Plants not retained for specific purposes were discarded at this time. Plant emergence and survival values were calculated by dividing the number of plants per entry in each replication by the average number of plants of the same entry growing in uninfested flats and multiplying the quotient by 100. Hence, the calculated value was the percent of plants in infested flats compared to the number of plants resulting from an equal number of seeds planted in uninfested flats. Pre- and postemergence values were in turn calculated from adjusted emergence and final count values.

Plants selected for continued resistance studies were identified at not later than 2 weeks of age by placing toothpicks near them (Plate IV). First choice selections were the larger individual surviving plants in areas of apparent heavy infection.

EXPLANATION OF PLATE IV

The relative resistance of eight alfalfa varieties to *Pythium irregulare* as illustrated by seedling stands in infested sand.

Toothpicks (indicated by arrows) identify alfalfa plants selected for continued resistance studies.

PLATE IV

Early selection was necessary as all surviving plants outgrew the symptoms very rapidly.

Flats containing selected plants were irrigated every 2 weeks with a nutrient solution composed of 5 g. of Hyponex per gallon of water. Nitrogen fixing bacteria inoculum was sprinkled on these flats.

Evaluation of Testing Technics

PDA vs. PDB. *Pythium irregulare*, cultured on PDA and PDB was applied separately to flats of sand at rates of 2½ Petri plates and mycelium from 500 ml. respectively. Four replications from each of eight alfalfa varieties were planted in the infested sand. The analysis of variance was calculated for each test and the respective coefficients of variation (52) compared as a method of evaluating the two culturing methods.

Comparison of Sand and Soil When Irrigated With Nutrient Solution or Water. Four metal flats were each divided into quarters with aluminum foil. Two quarters were filled at random with sand and 2 with sterile Geary silty clay loam from the University Agronomy Farm. The sand and soil were infested with *P. irregulare* at the rate of 2 plates per flat.

Four rows containing 50 Cody seeds each were planted in each section. Upon emergence, 1 sand and 1 soil section per flat was watered with a nutrient solution prepared by mixing 5 g. of Hyponex in 1 gallon of tap water. The other two sections received only tap water.

Two weeks after planting, two judges ranked the four sections within each flat with respect to uniformity of stand. The most uniform section was ranked number 1. The data were analyzed according to the Friedman rank test (22).

Healthy vs. Diseased Plants as Sources of Resistance. Cody plants Py 20 to 37 were selected from *Pythium* infested soil to determine the best approach in selecting resistant plants. The even numbered selections were apparently healthy while the odd numbered selections were definitely diseased. The selected plants were transferred to the field, caged, and the two groups intercrossed separately. Seeds harvested from individual plants were planted in *Pythium* infested sand and the resulting seedling survival determined.

Effect of Seed Factors on Seedling Survival in Infested Sand

Size. Six replications, each containing five sizes (Table 4) of 1947 California produced Syn 1 Cody alfalfa seeds were planted in sand infested with *P. irregulare* at a rate of $1\frac{1}{2}$ plates per flat. The experiment was conducted in a growth chamber maintained at $60 \pm 2^{\circ}$F. with 12 hours of light (500 foot candles) per day.

Spacing. Because of the tendency of *Pythium* to move from plant to plant within rows, a study was undertaken to determine the optimum seeding rate for a given area to obtain maximum uniformity of seedling survival.

Cody alfalfa seeds were planted at rates of 1, 2, 5, and 10

seeds per inch in 10-inch rows spaced 2 inches apart in flats of sand infested with $1\frac{1}{2}$ plates of *P. irregulare*. Each entry was repeated 6 times.

Seedling survival from 10 seeds in each row was included in the evaluation. Row sections utilized for survival counts were selected at random.

Area of Production. Due to observed differences in *Pythium* resistance among seed lots within varieties, a study was initiated to determine the effect of the seed production area upon the reaction of seedlings grown in infested sand.

Six replications of 1960 produced certified Buffalo alfalfa seed from six states were planted in sand infested with *P. irregulare*. The experiment was repeated with four replications and an additional entry.

Seeds from seven lots of 1960 produced certified Buffalo alfalfa were received from Utah and a test performed as described above to study the reaction of seedlings originating from seeds of the same variety produced in a localized area of one state.

Selection for Resistance to *Pythium*

Two Selection Cycles With Cody Alfalfa. Twelve Cody plants were selected from flats of soil infested with *P. irregulare*. Each plant was vegetatively increased and two representatives of each clone were transferred to the field and caged prior to flowering. The clones were intercrossed by alternately tripping florets randomly with a toothpick without emasculation. Suffi-

cient clonal material of Py 1, 2, 3, 4, 10, 11, and 12 survived in the cutting beds to provide 18 single plant replications of each which were arranged in a randomized complete block design in an 18 foot-square plot. The plot was caged and the plant inter-pollinated by honeybees.

Seeds from plants included in the intercross and polycross were planted in _Pythium_ infested sand. Five surviving seedlings were selected from each of Py 2, 3, and 11 of the 7-clone poly-cross. The five plants from each clone were hand intercrossed in the greenhouse. Also included in separate intercrosses in the greenhouse were: three plants each of clones Py 2, 3, and 11 (original selections); and two plants each of clones Py 1, 2, 3, 4, 10, 11, and 12.

A three generation comparison of seedling reaction to _Pythium_ was conducted by bulking and planting equal numbers of seeds from plants representing each of two selection cycles and Cody in in-fested sand. An additional entry, bulked seeds from the Py 2, 3, and 11 intercross, were also included. Included seeds were pro-duced in 1960 and except for Cody were produced in the greenhouse.

Seeds produced by individual plants from each of the five plant intercrosses of Py 2, 3, and 11, their respective parents, and Cody were planted in _Pythium_ infested sand and the seedling survival determined.

One Selection Cycle With Eight Alfalfa Varieties. Five se-lections each from eight alfalfa varieties were made from sur-viving plants in _Pythium_ infested sand of the PDA vs. PDB test (Table 1) and the 16 variety test (Table 22). Two such selections

from the PDA vs. PDB test are shown in Plate IV. Selected seedlings were transferred to 4-inch pots and intercrossed in the greenhouse.

Equal numbers of seeds from each plant included in a varietal intercross were bulked and planted in Pythium infested sand adjacent to rows of seeds from the parental seed lot with the exception of Buffalo. Original Buffalo selections were made from seedlings derived from seed lot F.C. 32984; however, because the seed supply from this lot was depleted Buffalo F.C. 33557 seeds were substituted in later studies. Tests, each including four replications and utilizing a split-plot design, were conducted at 60° F., 82° F., and under greenhouse conditions.

Seeds produced by individual plants of the Cody and Moapa intercrosses were planted in Pythium infested sand with those of their respective parental selections and varieties and the resulting seedling reactions compared.

Reaction of Alfalfa Varieties and Selections to Pythium Isolates

Six replications each of four alfalfa varieties were planted in sand infested with three Pythium species. A split-plot design was utilized with Pythium species serving as main plots and alfalfa varieties as sub-plots. The study was conducted in growth chambers at 65° ± 2° F.

In a similar study intercross seeds of five plants selected from Cody alfalfa and expressing varying degrees of resistance to P. irregulare were planted in the greenhouse in flats of sand infested with four isolates representing three Pythium species.

Evaluation of Alfalfa Species and Varieties for Resistance to *Pythium*

Seeds of 21 alfalfa varieties included in two tests were planted in sand infested with *P. irregulare* and their relative seedling reaction compared.

In another study seeds from 50 *Medicago sativa* and *M. falcata* introductions including diploids and tetraploids supplied by the Regional Plant Introduction Station at Ames, Iowa, were planted in sand infested with *P. irregulare* and their relative seedling reaction compared with that of previously tested varieties.

Because of the limited number of seeds received and the variation in seed sizes characteristic among the entries tested, the sizes of the seeds planted were determined by passing them through dodder sieves of various diameters as shown in Table 4. This afforded further opportunity to study the relationship between seed sizes and the relative *Pythium* tolerance of seedlings derived from them.

The harvest dates were supplied with many of the entries and recorded to gain information regarding the importance of seed age on the establishment of alfalfa stands under *Pythium* infestation.

RESULTS AND DISCUSSION

Evaluation of Testing Technics

PDA vs. PDB. It was proposed that uniformity of kill might be improved if inoculum free of the nourishment provided by PDA were used. This was accomplished by propagating the organism in

PDB and using washed mycelium blended with water as inoculum.

In comparing the two methods however, the coefficients of variation for seedling survival data were 19 and 34 percent where inoculum was increased in PDA and PDB respectively (Table 1). Increased variability with PDB might be partially attributed to the lower rate of seedling survival obtained in that test.

Table 1. Survival of seedlings of eight alfalfa varieties grown in sand infested with P. irregulare cultured on PDA and PDB.

		Plant survival[1]			
		PDA		PDB	
Variety	F.C. no.	Angle[2]	% of controls	Angle	% of controls
Buffalo	32984	36.44	35.25	19.35	12.20
Rhizoma	34035	34.20	31.90	32.76	29.70
Moapa	33926	24.70	17.60	22.72	15.95
Vernal	31983	22.83	15.18	19.38	12.35
Ranger	33347	19.06	11.90	13.56	5.95
Cody[3]	---	13.37	10.55	16.75	8.85
Lahontan	33718	12.28	4.78	13.84	7.55
Rambler	34135	10.56	3.12	12.50	5.00
LSD 5%		5.99		9.47	
LSD 1%		8.16		12.89	
CV		19%		34%	

1/ % of stand obtained in uninfested sand. Average of four replications.

2/ Arcsin transformation.

3/ 1957 California produced Syn 1.

On the basis of results yielded by this study and other factors, as the time and effort required for incubation and preparation of inoculum, PDA was considered the preferable medium.

Comparison of Sand and Soil When Irrigated With Nutrient Solution or Water. Rankings by two judges presented in Table 2 and analyzed by the Friedman Rank Test (22) yielded a chi-square

value of 7.35 (52). Thus the test revealed significant differences at the 10 percent level in uniformity among seedling stands of Cody alfalfa grown in Pythium infested sand and soil at two nutrition levels. On the basis of information gathered by the uniformity test and ease of handling, sand appeared the preferable plant medium.

Table 2. The effects of sand and soil when irrigated with nutrient solution or water upon the uniformity of stand of Cody alfalfa seedlings as ranked by two judges.

		Rank 1/			
		Sand 2/		Soil	
Replications	Judge	Nutrient sol.	Water	Nutrient sol.	Water
1	A	2	1	4	3
	B	2	1	4	3
2	A	1	2	4	3
	B	1	2	4	3
3	A	2	1	3	4
	B	4	3	1	2
4	A	1	2	4	3
	B	4	2	3	1
Total		17	14	27	22
Mean		2.12	1.75	3.38	2.75

1/ Number 1 was most uniform.
2/ Sand and soil were infested with P. irregulare.

Regardless of the care exercised in preparing and infesting either sand or soil there were areas of increased kill within the flats. However, these areas were more common and became larger in soil by the increased tendency of the organism to move from plant to plant within rows.

A higher percent of seedling survival resulted in sand than

in soil at equal levels of Pythium infestation. Neither uniformity of test nor seedling survival appeared greatly affected by irrigation with either nutrient solution or water.

Healthy vs. Diseased Plants as Sources of Resistance. Data regarding progeny survival in infested sand failed to indicate a difference between healthy and diseased alfalfa plants as sources of resistance to Pythium (Table 3).

Table 3. Survival, in Pythium infested sand, of seedling progenies derived from intercrosses of healthy Cody alfalfa plants compared to those derived from diseased plants.

Healthy 1/		Diseased 2/	
Selection	Plant survival 3/ (% of controls)	Selection	Plant survival 3/ (% of controls)
Py 36	58.24	Py 35	77.42
Py 24	57.45	Py 31	61.51
Py 30	52.94	Py 37	57.54
Py 22	41.73	Py 27	43.82
Py 28	36.29	Py 33	37.46
Py 34	35.25	Py 25	17.22
Py 26	31.23		
Mean	44.73		49.16

1/ Parental plants were selected as healthy appearing seedlings from Pythium infested sand.

2/ Parental plants were diseased but survived Pythium infection.

3/ Average of six replications. Survival of included Cody seedlings was 32.18%. Differences in seedling survival were not significant at the 5% level.

The failure of the F test to denote significant differences in seedling survival among entries appeared due to erratic post-emergence damping-off. Observations of some selections that reacted rather consistently in the six replications gave evidence that wide levels of Pythium resistance were represented by selections within each group.

Plants not listed in Table 3 either died or failed to produce sufficient seed for the test.

Effect of Seed Factors on Seedling Survival in Infested Sand

Size. Highly significant differences in favor of the larger seeds were found in the ability of seedlings derived from Cody alfalfa seeds of five sizes (Table 4) to survive in *Pythium* infested sand. The reason for the reduced seedling stand (65 percent) produced by the largest seeds (size 1) in the uninfested control flats was not determined. A close examination of some of these seeds under 10-power magnification failed to reveal any mechanical damage.

Similar reactions were expressed by seedlings derived from seeds of sizes 2 and 3 which were the predominating sizes of seeds produced by tetraploid alfalfa plants under normal conditions. In order to minimize seed size effects only seeds of sizes 2 and 3 were used in later studies when possible and applicable.

Size-4 seeds were least affected by pre-emergence damping-off. However, seedlings produced by size-4 seeds were smaller than those derived from larger seeds and relative few survived postemergence damping-off.

Size-5 seeds produced highly significantly less and noticeably weaker seedlings than did the larger seeds, with only a single plant surviving from the 300 size-5 seeds planted in *Pythium* infested sand.

Table 4. Reaction of Cody alfalfa seedlings derived from seeds of five sizes planted in Pythium infested sand.

Seed size No.	Seed size Dia. in mm.	% stand in controls	damping-off Pre.	damping-off Post.	Plant survival[1/] % of controls	Plant survival[1/] Angle[2/]
1	2.007-1.651	65	67.18	18.46	14.36	21.38
2	1.651-1.448	82	59.45	32.01	8.54	16.55
3	1.448-1.270	80	55.26	33.49	11.25	18.38
4	1.270-1.016	90	49.43	46.12	4.45	8.58
5	1.016-0.838	50	87.94	11.06	1.00	1.92
LSD 5%						7.76
LSD 1%						10.58

1/ Average of six replications.

2/ Arcsin transformation.

Spacing. The survival of Cody seedlings in Pythium infested sand was significantly affected by the seeding rate (Table 5). The mean plant emergence from 10 seeds of 7.7, 7.0, 8.0, and 8.2 at planting rates of 10, 5, 2, and 1 seeds per inch respectively approached the average emergence expected in uninfested sand. This indicated that pre-emergence damping-off was of minor importance in the test. Postemergence damping-off greatly reduced seedling survival at the higher seeding rates. Therefore, seedling survival increased as the distance between seeds increased.

The variance likewise increased with increased seeding rates, as the number of survivors from 10 Cody seeds ranged from 0 to 9 at the 10-seeds-per-inch rate. This increased variance was due to erratic postemergence damping-off caused by the pathogen moving from plant to plant within some rows.

It was difficult to make comparisons as there was little or no kill at the wider seed spacings, consequently, the resulting

variances were smaller.

Table 5. Survival of Cody alfalfa seedlings derived from seeds planted at four seeding rates in sand infested with *P. irregulare*.

	Surviving plants from 10 seeds Replications							
Seeds per inch	1	2	3	4	5	6	x	s
10	2	1	3	9	0	4	3.17	3.17
5	6	10	8	4	5	7	6.67	2.16
2	7	7	8	10	10	7	8.17	1.47
1	8	8	8	7	10	9	8.33	1.03
LSD 5%							2.88	

Area of Production. A lot of California-produced alfalfa seed produced considerably fewer surviving seedlings in *Pythium* infested sand than lots of certified Buffalo alfalfa seed produced in five other states in 1960 (Table 6). Because of high variability due to erratic postemergence damping-off, the test was repeated including seeds from Idaho. Again the California seed produced the smallest number of surviving seedlings although not significantly different from that of New Mexico and Texas produced seed.

An analysis of variance of seedling survival data from seeds representing seven lots of certified Buffalo seed produced in a localized area of Utah in 1960 and planted in infested sand failed to yield a significant F value (Table 7). However, the multiple t test (20) revealed significant differences at the five percent level.

In search of an explanation for the wide differences found

Table 6. Survival in *Pythium* infested sand of seedlings derived from certified Buffalo alfalfa seeds produced in seven states in 1960.

State	Designation	% stand in controls	% damping-off 1/		Plant survival 2/	
			Pre.	Post.	First test	Second test
Idaho	Lot A-2264	94	23.9	6.9	----	69.2
Utah	Lot M-2526-B	94	20.6	14.4	50.3	64.9
Oklahoma	----------	82	19.2	18.8	40.0	61.9
Kansas	# 4186	96	26.6	13.8	49.0	59.6
Texas	Mark 74	92	23.9	19.7	40.0	56.4
New Mexico	# 345	91	29.2	22.9	42.6	47.9
California	# 36793 File 19	91	35.2	27.8	25.2	36.9
LSD 5%					16.2 3/	17.6

1/ Includes only data from second test.

2/ % of stand obtained in uninfested controls. Average of six and four replications for tests one and two, respectively.

3/ F value not significant at the 5% level.

in seedling stands in the controls (Tables 6 and 7) seeds from lots represented in both tests were closely examined under 10-power magnification. Eight and 12 percent of the seeds examined from lots M-2571-B and M-2570-B (Table 7) respectively had cracked seed coats. Similar damage to other seed lots was less than two percent. The reason for the poor stand in the controls secured from the Oklahoma seed (Table 6) was not determined.

Table 7. Survival in *Pythium* infested sand of seedlings derived from certified Buffalo alfalfa seeds produced in Utah in 1960.

Seed lot	% stand in controls	% damping-off		Plant survival1/
		Pre.	Post.	
M-2526-B	88	8.3	17.1	74.6
M-2501-B	94	20.3	12.3	67.4
O-135-B	96	19.6	14.4	66.0
M-2570-B	80	22.0	20.8	57.2
M-2512-B	86	28.3	14.9	56.8
M-2508-B	92	21.7	26.0	52.3
M-2571-B	90	33.3	15.1	51.6
LSD 5%				18.4 2/

1/ % of stand acquired in uninfested controls. Average of six replications.

2/ F value not significant at the 5% level.

Selection for Resistance to *Pythium*

Two Selection Cycles With Cody Alfalfa. Cody alfalfa selections Py 2, 3, and 11 were significantly more tolerant to *Pythium irregulare* than Cody as indicated by the relative seedling survival of their respective polycross progeny in *Pythium* infested sand (Table 8).

Progeny survival data from a test involving seeds of Cody

Table 8. Seedling tolerance in *Pythium* infested sand of intercross and polycross progenies derived from Cody plants previously selected from *Pythium* infested sand.

Polycross (7 clones)1/					Intercross (12 clones)2/			
	% damping-off		Plant survival3/			% damping-off		
Selection	Pre.	Post.	% of controls	Angle4/	Selection	Pre.	Post.	Plant survival (% of controls)
Py 11	34.7	21.8	43.6	41.0	Py 2	20.0	31.3	48.7
Py 3	42.0	23.6	34.4	35.8	Py 1	32.6	19.7	47.7
Py 2	44.7	23.2	32.1	34.2	Py 5	31.2	32.4	36.4
Py 4	59.8	16.4	23.8	28.3	Py 12	52.1	15.3	32.6
Py 10	61.7	15.7	22.6	28.2	Py 8	52.8	15.0	32.1
Py 1	43.7	34.3	22.0	27.4	Py 6	32.6	36.5	30.9
Cody5/	70.2	14.5	15.3	22.1	Py 3	58.0	11.4	30.7
Py 12	72.6	12.2	15.2	20.5	Py 7	45.3	24.3	30.4
LSD 5%				9.7	Py 10	55.1	17.2	27.8
LSD 1%				12.9	Py 4	69.7	13.3	17.0
					Cody	75.3	12.1	12.6
					LSD 5%			11.8
					LSD 1%			15.8

1/ Seed resulted from honeybee pollination of caged plants.

2/ Seed resulted from hand pollination of caged plants in the field. Clones Py 9 and 11 failed to produce sufficient seed to be included in the test.

3/ Six replications were included in each test.

4/ Arcsin transformation.

5/ 1957 California produced Syn 1 seeds.

and those produced by intercrossing Cody selections Py 1 to 12 indicated that all included selections except Py 4 were significantly more tolerant to Pythium irregulare than was Cody (Table 8).

Seedling survival from first and second cycle selections of Cody alfalfa did not differ significantly but survival from both significantly exceeded that of Cody (Table 9).

Table 9. Reaction in Pythium infested sand of Cody alfalfa compared to that of seedlings representing two selection cycles from Cody.

Selection cycle	% damping-off		Surviving plants (% of controls)
	Pre.	Post.	
1 1/	6.2	2.7	91.1
2 2/	3.4	10.0	85.7
1 3/	3.6	19.8	76.6
Cody 4/	4.9	32.8	62.3
LSD 5%			17.5
LSD 1%			23.8

1/ Seeds resulted from intercrossing clones Py 1, 2, 3, 4, 10, 11, and 12.

2/ Seeds resulted from five plant intercrosses from each of clones Py 2, 3, and 11.

3/ Seeds produced by intercrossing clones Py 2, 3, and 11.

4/ 1960 Kansas produced Syn 1 seeds.

Three plants each of clones Py 2, 3, and 11 (original selections) were intercrossed and equal numbers of seeds from each bulked and included in the test (Table 9). It was proposed that by intercrossing these three supposedly most resistant clones that the resulting progeny would be more tolerant to Pythium than those from the 7-clone intercross. However, the Pythium tolerance of the intercross progeny from the two groups was not

significantly different.

A study of the reaction of progeny from each of the five plant selections from Py 2, 3, and 11 revealed (Table 10) that only 2 of the 15 second cycle selections, Py 11-1 and Py 11-2, produced significantly more surviving seedlings than their respective parents. In this instance the parental selection, Py 11, and Cody did not differ significantly with respect to Pythium tolerance.

The poor performance of Py 3 as shown in Table 10 was explainable on the basis of results from other tests. In the 12-clone intercross test (Table 8) highly significantly fewer Py 3 progeny survived Pythium infestation than those of Py 2 and 1. Also, seedling survival data (Table 16) from a study of the reaction of Cody selections Py 1, 2, 3, 11, and 12 of the 7-clone intercross to three Pythium isolates revealed that Py 2, 11, and 1 were significantly more tolerant to Pythium than were Py 3 and 12. These data further suggested that Py 1 should have replaced Py 3 in the extended selection studies.

In surveying the pre- and postemergence data involved in this study some interesting observations were noted. Pre-emergence kill (Table 8) increased with the susceptibility of entries at a greater rate than the corresponding decrease in seedling survival. Therefore, postemergence damping-off destroyed fewer plants of the more susceptible than of the more tolerant entries. However, the trend was reversed in the three generation comparison study (Table 9) as postemergence damping-off losses exceeded those from pre-emergence damping-off.

Table 10. Seedling reaction in _Pythium_ infested sand of Cody alfalfa, Py 2, 3, and 11 and intercross progenies of five plants previously selected from each of Py 2, 3, and 11.

Py 2				Py 3				Py 11			
	% damping-off				% damping-off				% damping-off		
Selection	Pre.	Post	% survival 1/	Selection	Pre.	Post	% survival	Selection	Pre.	Post	% survival
Py 2 2/	25.4	11.6	63.1	Py 3-2	18.2	6.1	75.7	Py 11-1	3.1	1.5	95.4
Py 2-5	27.7	10.8	61.5	Py 3	21.1	5.3	73.6	Py 11-2	6.5	2.2	91.3
Py 2-3	33.3	8.5	58.2	Py 3-1	13.2	13.6	73.2	Py 11-5	5.7	9.5	84.8
Py 2-4	43.7	1.2	55.1	Py 3-4	16.7	10.5	72.8	Py 11-3	3.6	13.8	82.6
Py 2-1	35.1	24.6	40.3	Cody	9.9	21.5	69.6	Py 11-4	13.6	5.2	81.2
Py 2-2	54.4	5.6	40.0	Py 3-5	27.8	3.5	68.7	Cody	10.8	13.8	75.4
Cody 3/	28.8	36.1	35.1	Py 3-3	28.3	17.3	54.4	Py 11	23.2	4.0	72.8
LSD 5%			15.1				ns.				13.5
LSD 1%			20.3				ns.				ns.

1/ % of stand in uninfested sand. Each test included six replications.

2/ Seeds from Py 2, 3, and 11 resulted from an intercross including Py 1, 2, 3, 4, 10, 11, and 12.

3/ 1957 California produced Syn 1 Cody seeds.

<u>One Selection Cycle With Eight Alfalfa Varieties</u>. In a test conducted under greenhouse conditions significant gains in tolerance to *Pythium* were made in Ranger, Vernal, Lahontan, and Cody alfalfa varieties (Table 11).

Largest gains in resistance were made in those varieties which themselves exhibited low levels of resistance. Seedling survival of Rhizoma, the most resistant variety, was less than that of intercross progeny from Rhizoma plants selected for *Pythium* resistance. Highly significant differences in *Pythium* resistance existed among the included varieties.

At temperatures maintained near 82° and 60° F. in growth chambers significant gains in *Pythium* tolerance was acquired in six of the eight included varieties (Table 12. and Fig. 1). Progeny survival of first cycle selections from Rhizoma and Moapa failed to significantly surpass that of their respective parental varieties. A loss of *Pythium* resistance, although not significant, was recorded at both 82° and 60° F. with Moapa.

Again, as recorded at greenhouse temperatures (Table 11), *Pythium* resistance, as the result of selection, increased as the levels of resistance of the respective varieties decreased.

The inability to increase *Pythium* resistance in the more resistant varieties was not determined although the influence of the resistance level of the populations from which selections were made offers a partial explanation. Selections were made from infested flats where all varieties were subjected to the same levels of infestation. Therefore, selections were made from the most resistant 5 percent (Tables 1 and 22) of the Rambler plants,

Table 11. Seedling tolerance in *Pythium* infested sand of eight alfalfa varieties compared to that of intercross progeny of plants selected from them. Test was conducted in the greenhouse.

		% stand in controls		% damping-off				Plant survival 1/ (% of controls)		
				Variety		Intercross				
Variety	F.C. no.	Variety	Intercross 2/	Pre.	Post.	Pre.	Post.	Variety	Intercross	% gain 3/
Rhizoma	34035	94	94	14.44	6.84	23.33	8.59	78.72	68.08	- 10.64
Moapa	33926	98	96	19.39	15.29	21.88	7.30	65.32	70.82	5.50
Buffalo	33557	98	96	30.21	19.79	25.58	22.34	50.00	52.08	2.08
Ranger	33347	94	86	33.33	18.79	19.05	7.70	47.88	73.25	25.37
Vernal	31983	96	84	32.29	20.83	17.07	12.71	46.88	70.22	23.34
Lahontan	33718	100	90	38.00	19.00	23.26	16.74	43.00	60.00	17.00
Cody	---	94	86	46.81	17.01	32.50	4.70	36.18	62.80	26.62
Rambler	34135	98	94	46.81	21.59	43.48	17.14	31.60	39.38	7.78
LSD 5%								21.73		
LSD 1%								29.58		

1/ Average of four replications.

2/ Intercross progeny of five plants selected from infested sand. Equal numbers of seeds from each plant in an intravarietal intercross were bulked.

3/ Gains or losses of less than 20.63% are not significant at the 5% level.

Table 12. Seedling survival in *Pythium* infested sand of eight alfalfa varieties compared to that of intercross progeny of plants selected from them. Test was conducted in growth chambers at controlled temperatures.

		82° F. Mean survival1/ (% of controls)		60° F. Mean survival (% of controls)		Average of 2 temps.		
Variety	F.C. no.	Variety	Intercross2/	Variety	Intercross	Variety	Intercross	% gain3/
Rhizoma	34035	75.52	83.72	66.69	69.05	71.10	76.39	5.29
Moapa	33926	75.02	74.50	48.95	47.00	61.99	60.75	- 1.24
Lahontan	33718	55.10	64.28	43.48	61.12	49.29	62.70	13.59
Vernal	31983	56.40	92.85	38.00	53.58	47.20	73.21	26.01
Buffalo	33557	53.40	63.28	36.95	58.88	45.18	61.08	15.90
Rambler	34135	52.05	71.75	23.95	55.82	38.00	63.79	25.79
Ranger	33347	55.00	75.00	19.02	43.20	37.01	59.10	22.09
Cody	---	32.98	71.75	23.35	53.65	28.16	62.70	34.54
LSD 5%		19.92		16.52				
LSD 1%		27.11		22.48				

1/ Average of four replications at each temperature.

2/ Intercross progeny of five plants selected from infested sand. Equal numbers of seeds from each plant in an intravarietal intercross were bulked.

3/ Gains or losses of less than 12.53% and 16.57% are not significant at the 5% and 1% levels respectively.

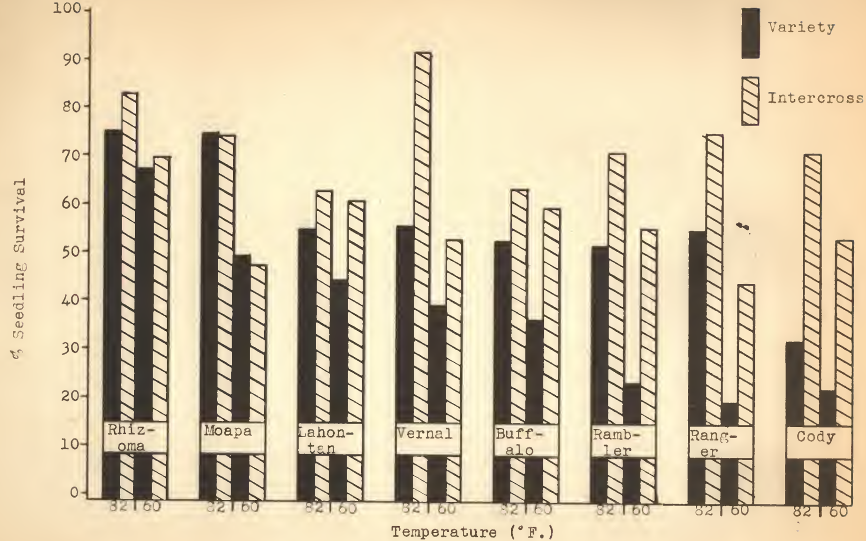

Fig. 1. Relative seedling survival in Pythium infested sand at two temperatures of eight alfalfa varieties and intercross progeny of plants selected from them.

and from the most resistant 35 percent of the Rhizoma plants.

The analysis of variance (Table 13) revealed a significant temperature x variety interaction. However, the systems (variety + intercross) x temperature interaction was nonsignificant which offered encouragement from the standpoint of resistance breeding. Further evidence of the independence of temperature upon the accumulation of Pythium resistance in alfalfa by selection was the nonsignificant varieties x temperatures x systems interaction.

Pre- and postemergence damping-off data (Table 14) revealed that under greenhouse conditions about twice as many varietal seedlings were killed by pre- as by postemergence damping-off. The same trend existed also with respect to the bulked intercross seedlings except it appeared more erratic. Gains made in resistance to Pythium by selection relative to increased tolerance to pre- versus postemergence damping-off were not apparent.

Comparison of pre- and postemergence damping-off values at 82° and 60° F. (Table 12) revealed that seedling survival was approximately 20 percent higher at 82° than 60° F. Temperature drastically affected the balance between pre- and postemergence kill but made little difference in the final outcome relative to increasing Pythium tolerance by selection. Pre-emergence damping-off accounted for nearly 75 percent of the kill at 60° F. while postemergence damping-off predominated at 82° F.

The temperature effect on the damping-off of seedlings is well known (2,5,6,7,14,15,21,25,30,31,33,34,39,42,49,51,58), however, the temperature effect found in this study with respect to total seedling losses and its relative effect on pre- and post-

Table 13. Analysis of variance for data regarding seedling survival in _Pythium_ infested sand of eight alfalfa varieties compared to that of intercross progeny of plants selected from them.

Source of variation	d.f.	Ss	Ms	F	Sig.
Temperatures	1	12006.688	12006.688	68.01	***
Reps : Temps (a)	6	1059.313	176.552		
Systems	1	10051.848	10051.848	70.70	***
S x T	1	.009	.009	.00	ns.
S x R:T (b)	6	853.052	142.175		
Varieties	7	9126.624	1303.803	8.24	***
V x T	7	2696.774	385.253	2.44	*
V x S	7	3886.297	555.185	3.51	**
V x T x S	7	926.232	132.319	.84	ns.
Error (c)	84	13284.352	158.147		

Table 14. Pre- and postemergence damping-off in *Pythium* infested sand of eight alfalfa varieties compared to that of intercross progeny of plants selected from them.

		% Damping-off							
		82° F.				60° F.			
		Variety		Intercross		Variety		Intercross	
Variety	F.C. no.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.
Rhizoma	34035	7.42	17.06	2.45	13.83	32.15	1.17	30.50	0.45
Moapa	33926	3.10	21.88	6.25	19.25	28.12	22.93	35.42	17.58
Lahontan	33718	18.35	26.55	7.78	27.94	42.20	14.32	30.68	8.20
Vernal	31983	4.55	39.05	- 3.75 1/	10.90	45.90	16.10	34.20	11.22
Buffalo	33557	10.00	36.60	11.45	25.27	37.78	25.27	27.28	13.84
Rambler	34135	24.48	23.47	14.15	14.10	55.30	20.75	31.38	12.80
Ranger	33347	12.82	32.18	11.92	13.08	52.45	28.53	40.92	15.88
Cody	---	28.25	38.77	0.10	28.15	53.38	23.27	30.78	15.57
Mean		21.97	29.44	6.29	19.06	43.41	19.04	32.64	11.94

1/ Average seedling emergence in infested sand was 103.75% of that in uninfested controls.

emergence damping-off did not agree with previous work. Buchholtz (5,6) found less damping-off of alfalfa at 9° C. than at 20 to 25° C. Halpin et al. (30) and Hanson (31) obtained best stands with small seeded legumes, including alfalfa, at lower temperatures. Gerdemann (23) found red clover more susceptible to pre-emergence damping-off in warm (70 to 75° F.) than in cold soil (45 to 55° F.).

The influence of temperature on the moisture content of the sand offered a possible explanation for the relatively low level of pre-emergence damping-off as it was more difficult to keep the sand moist at the 82° F. temperature.

Temperature effects on seedling emergence and *Pythium* symptoms were also noted. At 82° F. seedling emergence began in the controls on the second day following planting and by the third day a 50 percent stand was acquired in the infested sand. At 60° F. little seedling emergence occurred prior to the fourth day.

First postemergence damping-off, which under greenhouse temperatures occurred between 5 and 7 days after planting, occurred on the fourth day in the 82° F. chamber and on the ninth day at 60° F.

In the warm chamber damped-off plants soon died. In the cold chamber, due to the low temperature and high relative humidity, many damped-off plants formed adventitious roots above the lesions and continued to grow.

Tests were conducted to investigate the relative *Pythium* tolerance of individual selections included in some of the intravarietal intercrosses. Because of limited time and space only selections from Cody and Moapa were tested. These varieties were

chosen as they represented varieties in which the most and the least progress respectively had been made in increasing resistance to Pythium. In addition to the five selections, seeds from the parental seed lot and those produced in 1960 by plants from another seed lot within the same variety were included (Table 15).

Table 15. Seedling reaction in Pythium infested sand of two alfalfa varieties compared to that of intercross progenies of plants previously selected from each variety in Pythium infested sand.

Cody				Moapa			
Selection	% damping-off Pre.	% damping-off Post.	% survival 1/	Selection	% damping-off Pre.	% damping-off Post.	% survival
C 5	8.8	7.5	83.7	Moapa 2/	7.4	22.3	70.3
C 3	8.1	8.2	83.7	M 3	24.8	5.8	69.4
C 1	13.0	13.3	73.7	M 2	19.2	12.8	68.0
C 4	7.0	24.7	68.2	M 1	16.7	16.6	66.7
Cody 3/	10.2	29.0	60.8	Moapa 4/	20.7	12.6	66.7
C 2	9.0	34.1	56.9	M 5	17.7	17.0	65.3
Cody 5/	14.4	33.8	51.8	M 4	22.2	17.9	59.9
LSD 5%			18.9				ns.
LSD 1%			25.4				

1/ % of stand acquired in uninfested sand. Each test included six replications.

2/ F.C. 33926. Seed lot from which Moapa selections were made.

3/ 1960 Kansas produced Syn 1 seeds.

4/ 1960 California produced seeds.

5/ 1957 California produced Syn 1 seeds. Seed lot from which Cody selections were made.

Progeny survival from selections C 5, 3, and 1 of Cody was significantly greater than that from the parental Cody seed lot and C 5 and 3 were highly significantly greater. The mean seedling survival of progeny produced by the five Cody selections was 73 percent and that of the parental Cody 52 percent. This

was an increase in resistance of 22 percent which was comparable to gains found in previous tests (Tables 11 and 12).

Differences in seedling survival found among progeny of seeds of each of the five plants selected from Moapa and the parental seed lot were nonsignificant. The mean seedling survival of progeny from plants selected from Moapa was 66 percent and that from the parental Moapa seed lot 70 percent which also compared favorably with those previously attained (Tables 11 and 12).

Reaction of Alfalfa Varieties and Selections to *Pythium* Isolates

Five Cody Selections x Three Pythium Isolates. Polycross progeny of five Cody plants representing various degrees of resistance to isolate A-21-56 of *P. irregulare*, reacted in the same manner to isolates W-2 of *P. irregulare* and W-1 of *P. ultimum* (Table 16). Highly significant differences were found both in *Pythium* tolerance expressed among the five selections and in the amount of damping-off incited by the *Pythium* isolates represented (Table 17).

Isolate I-1 of *P. debaryanum* was included in the study but due to its high virulence less than 1 percent seedling emergence resulted in sand infested with this isolate. Therefore, these data were not included in the analysis.

Isolates W-2 of *P. irregulare* and W-1 of *P. ultimum* did not differ significantly in their relative pathogenecity; however, both isolates produced highly significantly more damping-off than isolate A-21-56 of *P. irregulare* (Table 16).

Cody selections Py 2, 11, and 1 were significantly more re-

sistant to the Pythium isolates than were Py 3 and 12.

Table 16. Reaction of five selections from Cody alfalfa to three Pythium isolates as expressed by the survival of their intercross progeny in infested sand.

	Mean plant survival[1] (% of controls)			
	P. irregulare		P. ultimum	Selection
Selection[2]	A-21-56	W-2	W-1	mean[3]
Py 2	74.20	37.10	33.05	48.12
Py 11	65.75	32.10	35.90	44.58
Py 1	66.12	24.27	31.02	40.47
Py 3	56.52	17.92	23.62	32.69
Py 12	51.43	20.58	19.88	30.63
Mean[4]	62.80	26.39	28.69	

1/ Average of four replications.

2/ Pys 1, 2, 3, 4, 10, 11, and 12 were included in the intercross.

3/ Differences between selection means of greater than 6.33 and 8.42 are significant at the 5% and 1% levels respectively.

4/ Differences between Pythium isolate means of greater than 9.59 and 13.65 are significant at the 5% and 1% levels respectively.

Table 17. Analysis of variance for data regarding the reaction of five Cody selections to three Pythium isolates.

Source of variation	d.f.	Ss	Ms	F	Sig.
Replications	5	971.27	194.25		
Pythium isolates	2	24939.54	12469.77	44.85	***
Error (a)	10	2780.36	273.04		
Selections	4	4068.31	1017.08	11.27	***
Sel x isolates	8	466.25	58.28	.65	ns.
R x I + R x S x I (b)	60	5416.40	90.27		

Pre- and postemergence data from this study (Table 18) indicated that differences in resistance existing among the Cody selections were due to their relative ability to survive preemergence damping-off as postemergence damping-off remained

Table 18. Pre- and postemergence damping-off data from intercross progeny of five Cody alfalfa selections in flats of sand separately infested with three *Pythium* isolates.

	% damping-off							
	P. irregulare				*P. ultimum*			
	A-21-56		W-2		W-1		Mean	
Selection	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.
Py 2	14.27	11.53	53.36	9.54	54.73	12.22	40.79	11.10
Py 11	23.02	11.23	50.79	17.11	45.24	18.86	39.68	15.73
Py 1	17.70	16.18	65.71	10.02	49.24	19.74	44.22	15.31
Py 3	29.34	14.14	71.58	10.50	69.28	7.10	56.73	10.58
Py 12	32.61	15.96	67.39	12.03	68.12	12.00	56.04	13.33
Mean	23.39	13.81	61.77	11.84	57.32	13.98	47.49	13.21

rather constant.

Four Varieties x Three Pythium Species. Seedlings of four alfalfa varieties representing a wide range of resistance to P. irregulare reacted in a similar manner to two other Pythium species (Tables 19 and 20).

The lack of interaction between alfalfa varieties and selections and Pythium isolates suggested that resistance accumulated against specific Pythium species might also aid in preventing or lessening infection from other Pythium species.

Pythium species P. ultimum (W-1) and P. irregulare (W-2) were not significantly different in their ability to incite infection; however, both were highly significantly more pathogenic than P. debaryanum (58-15C).

Rhizoma produced significantly more surviving seedlings than Buffalo and Lahontan. All three were significantly more tolerant to the Pythium species than Du Puits. The Du Puits seeds were smallest and dark in color suggesting aging although the age was not known.

The order of seedling emergence in controls and infested sand alike was Lahontan, Buffalo, Rhizoma, and Du Puits which, with the exception of Buffalo, agreed with the order of increasing pre-emergence damping-off (Table 21). This would suggest, as found by Leach (42), that rapid seedling emergence decreases pre-emergence infection. However, any such advantage toward increased seedling survival appeared nullified by postemergence kill (Table 21). Buffalo, with by far the least amount of pre-emergence damping-off greatly exceeded other varieties in the amount of postemer-

Table 19. Reaction of four alfalfa varieties to three _Pythium_ species as expressed by seedling survival in infested sand.

		Mean plant survival						
		P. debaryanum		P. irregulare		P. ultimum		
Variety	F.C. no.	Angle1/	% of controls	Angle	% of controls	Angle	% of controls	Angle mean2/
Rhizoma	34035	53.64	64.13	34.62	33.55	29.34	24.32	39.20
Buffalo	32984	34.56	33.15	31.62	28.43	31.66	28.00	32.61
Lahontan	33718	37.59	37.80	28.57	23.63	28.29	23.63	31.48
Du Puits	24648	26.30	20.90	13.12	6.72	8.30	4.70	15.91
P. species mean3/		38.02		26.98		24.40		

1/ Arcsin transformation.

2/ Differences between the variety means of 6.40 and 8.52 are significant at the 5% and 1% levels respectively.

3/ Differences between the _Pythium_ species means of 5.54 and 7.38 are significant at the 5% and 1% levels respectively.

Table 20. Analysis of variance for data regarding reaction of four alfalfa varieties to three _Pythium_ species.

Source of variation	d.f.	Ss	Ms	F	Sig.
Varieties	3	5258.832	1752.944	19.11	***
Species	2	2514.510	1257.255	13.71	***
V x spp.	6	858.807	143.134	1.56	ns.
Replications	5	323.264	64.653	.70	ns.
Error	55	5044.103	91.711		

Table 21. Pre- and postemergence damping-off at 65° F. of four alfalfa varieties grown in sand infested with three *Pythium* species.

		% Damping-off							
		P. debaryanum		*P. irregulare*		*P. ultimum*		Mean	
Variety	F.C. no.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.
Rhizoma	34035	21.85	14.02	29.18	37.27	33.02	42.75	28.02	31.35
Buffalo	32984	.02	66.93	10.80	60.77	8.18	63.82	6.32	63.84
Lahontan	33718	20.40	41.80	43.25	33.12	25.52	50.85	29.72	41.92
Du Puits	24648	19.85	59.25	49.37	43.91	64.12	31.18	44.45	44.78
Mean		15.52	45.50	33.15	43.77	32.71	47.15		

gence losses incurred. Rhizoma, a hardy type adapted to northern growing conditions and thus characterized by slow emergence and lack of seedling vigor, was most tolerant to Pythium of the varieties tested.

Postemergence damping-off remained rather constant as measured by the means listed under each Pythium species in Table 21; therefore, relative survival in Pythium infested sand depended, as under greenhouse temperatures, upon the ability of seedlings to emerge in infested sand.

Postemergence damping-off accounted for relatively few plant deaths under greenhouse conditions (Table 18), but was the predominate form expressed at 65° F. (Table 21).

Evaluation of Alfalfa Species and Varieties for Resistance to Pythium

Twenty-one Varieties. Highly significant differences were found among 16 alfalfa varieties as determined by the relative tolerance of derived seedlings to Pythium irregulare (Table 22).

A wide range in seed viability was represented as measured by the percent of stands acquired in uninfested sand relative to the number of seeds planted. Reduced seed viability in some instances was no doubt the result of aging although the ages of the seeds planted were not known. The influence of seed viability upon resulting seedling survival was not clearly determined.

In general, the less viable seed lots produced a lower percentage of surviving seedlings in Pythium infested sand than did high quality seed (Table 22). An exception, Buffalo F.C. 32984,

Table 22. Seedling survival of 16 alfalfa varieties planted in Pythium infested sand.

Variety	F.C. no.	% stand in controls	% damping-off Pre.	% damping-off Post.	Plant survival[1] Angle[2]	Plant survival[1] % of controls
Buffalo	32984	75	34.7	25.4	36.48	35.90
Rhizoma	34035	91	54.6	12.1	34.84	33.34
Moapa	33926	91	60.8	15.0	28.55	24.18
Epley	---	93	71.0	9.1	25.99	19.92
Williamsburg	34041	74	70.3	9.3	25.50	20.37
Vernal	31983	90	70.0	9.0	24.41	20.97
Ranger	33347	84	78.6	7.5	20.19	13.89
Hairy Peruvian	33601	91	78.4	9.1	20.14	12.50
Atlantic	32954	91	79.1	9.0	19.29	11.87
Ladak	32566	81	82.7	5.7	19.17	11.65
Cody (Syn 1)	---	87	78.6	10.3	19.05	11.11
Narragansett	32768	76	86.0	6.0	12.19	8.02
Rambler	34135	76	92.1	4.1	9.78	3.75
Lahontan	33718	74	90.1	5.8	9.16	4.11
Zia	33969	80	93.3	3.3	8.58	3.42
Du Puits	24648	43	95.4	1.1	4.58	3.55
LSD 5%					7.76	
LSD 1%					10.30	

1/ Average of six replications.
2/ Arcsin transformation.

which produced only a 75 percent stand in the controls appeared the most resistant of the varieties tested.

Differences in the seedling vigor possessed among represented varieties were apparent in the uninfested controls where seedlings of nonhardy varieties, Epley, Moapa, and Hairy Peruvian were the first to emerge. However, this trait was not expressed in the infested flats and appeared of minor importance in aiding these nonhardy varieties in establishing seedling stands in _Pythium_ infested sand. The two most _Pythium_ tolerant were Buffalo and Rhizoma (Table 22) which are considered to be medium hardy and hardy varieties respectively. Thus, with nonhardy varieties, Moapa and Epley being the third and fourth most resistant varieties respectively indicated that _Pythium_ tolerance was not closely associated with seedling vigor or climatic adaptability.

Most seedling kill was expressed in the form of pre-emergence damping-off which destroyed 95 percent of the Du Puits seedlings (Table 22). Postemergence damping-off was of little consequence except in the more resistant varieties where it accounted for nearly 75 percent of Buffalo seedling losses.

The same trends were found in a similar experiment including five alfalfa varieties (Table 23). The beneficial effects of increased seedling survival in controls upon the procurement of seedling stands in _Pythium_ infested sand were apparent and preemergence damping-off data gave a reliable indication of subsequent seedling survival.

Fifty Plant Introductions. Seedling survival of 15 alfalfa Plant Introductions from 13 countries and Cody alfalfa did not

Table 23. Seedling survival of five alfalfa varieties planted in _Pythium_ infested sand.

Variety	F.C. no.	% stand in controls	% damping-off Pre.	% damping-off Post.	Plant survival1/ Angle2/	Plant survival1/ % of controls
Grimm	32124	94	68.8	16.3	22.58	14.90
Teton	34112	88	67.1	21.9	18.05	10.98
Nomad	34054	87	81.4	7.1	17.78	11.50
Caliverde	32158	69	85.3	7.9	13.25	6.77
Kansas Common	---	66	95.8	1.7	5.31	2.53
LSD 5%					8.73	
LSD 1%					11.90	

1/ Average of six replications.

2/ Arcsin transformation. Cody included in another test within same flats yielded a 12.6 % plant survival value.

differ significantly in sand infested with Pythium irregulare (Table 24).

Table 24. Seedling survival of 15 tetraploid Alfalfa Plant Introductions in sand infested with P. irregulare.

P.I. no.	Origin	Survival1/ (% of controls)
193291	Yugoslvaia	19.75
202291	Argentina	18.75
188868	Canada	18.50
206340	S. Africa	18.25
163107	India	17.75
189393	New Zealand	16.75
179368	Syria	16.50
199272	Portugal	16.50
190258	Algeria	16.00
212858	Alghan	15.25
183060	India	15.00
222731	Iran	14.50
183263	Arabia	13.50
179369	Syria	13.25
Cody (Syn 1)	---	13.00
170453	Turkey	12.75
LSD 5%2/		4.73

1/ Average of four replications.
2/ F value not significant at the 5% level.

Highly significant differences in seedling survival were found among seven M. falcata entries but none was more resistant to Pythium than Rhizoma F.C. 34035 (Table 25). The advantages of increased seed size upon the establishment of seedling alfalfa stands in Pythium infested sand were clearly illustrated. Differences in seed ages of two years among the represented varieties in the test (Table 25) were insufficient to indicate the effect of seed age upon the resulting seedling survival.

Seedling tolerance to Pythium by alfalfa Plant Introductions from several countries varied widely (Table 26). P.I. 255178 from

Table 25. Seedling survival of seven alfalfa Plant Introductions in sand infested with *P. irregulare*.

No.	Species	Origin	Seed harvest date	Seed size[1]	Survival[2] (% of controls)
Rhizoma					
F.C. 34035	media	Canada	1958[3]	2 & 3	68.78
P.I. 234817	falcata	Switzerland	1958	2 & 3	56.10
P.I. 234818	falcata	Switzerland	1958	2 & 3	54.35
P.I. 251831	falcata	Italy	1959	3,4,2	51.12
P.I. 251760	falcata x sativa	Italy	1959	3,4,2	42.00
P.I. 234815	falcata	Switzerland	1958	3	38.65
P.I. 235021	falcata	Switzerland	1958	3 & 4	29.58
P.I. 234787	falcata	Sweden	1958	4	3.30
LSD 5%					17.58
LSD 1%					23.92

1/ Diameter of seed as described in Table 4 in the descending order of predominance.

2/ Average of four replications.

3/ Date of harvest unknown. Seed was received February, 1958.

Table 26. Seedling survival of alfalfa Plant Introductions in sand infested with *P. irregulare*.

No.	Species	Origin	Seed harvest date	Seed size1/	Survival2/ (% of controls)
P.I. 255178	sativa	Poland	1959	3,2,4	74.42
Rhizoma F.C. 34035	media	Canada	19583/	2 & 3	63.82
P.I. 238152	sativa	Turkey	1958	3,2,4	62.78
P.I. 251688	falcata	Russia	1960	3 & 4	60.00
P.I. 251690	falcata	Russia	1960	3,2	45.75
P.I. 231731	falcata	USSR	1960	3,2,4	42.55
P.I. 204886	falcata	Turkey	1960	3,2,4	41.88
P.I. 206456	falcata	Turkey	1959	2 & 3	41.10
P.I. 251830	falcata	Austria	1960	3,4	39.28
P.I. 234816	falcata	Switzerland	1958	2 & 3	36.20
P.I. 251689	falcata	Russia	1960	3,2,4	36.15
P.I. 228152	falcata	USSR	1960	3,4	25.58
P.I. 258750	falcata	USSR	1960	4,3	17.10
P.I. 253445	falcata	Yugoslovia	1960	3	11.48
LSD 5%					17.09
LSD 1%					22.86

1/ Size of seeds as described in Table 4 in descending order of predominance.

2/ Average of four replications.

3/ Date of harvest unknown. Seed was received February, 1958.

Poland produced the highest level of seedling survival in sand infested with *P. irregulare*, of the included entries although not significantly higher than that produced by Rhizoma. The influence of seed size upon seedling survival was apparent while age of seed effects appeared insignificant (Table 26).

Tetraploids and diploids of both *M. sativa* and *M. falcata* species yielded highly significant differences in apparent *Pythium* tolerance (Table 27). Although diploids appeared to be less tolerant than tetraploids these differences could be explained on the basis of seed size. The differences of four years among the ages of seeds represented appeared of little consequence in determining seedling stands in *Pythium* infested sand.

Table 27. Seedling survival of tetraploid and diploid alfalfa Plant Introductions in sand infested with *P. irregulare*.

No.	Species	Ploidy	Origin	Seed harvest date	Seed size[1]	Survival[2] (% of controls)
P.I. 204458	falcata	tetraploid	Turkey	----	3	85.90
P.I. 204885	falcata	tetraploid	Turkey	1955	3	84.45
Rhizoma						
F.C. 34035	media	tetraploid	Canada	1958[3]	3	81.25
P.I. 204457	falcata	tetraploid	Turkey	1955	3	80.78
P.I. 204460	falcata	tetraploid	Turkey	1957	3	71.10
P.I. 206286	sativa	diploid	Turkey	1955	4	70.22
P.I. 172983	sativa	diploid	Turkey	----	4	69.30
P.I. 206454	sativa	diploid	Turkey	1955	4	67.68
P.I. 204459	falcata	tetraploid	Turkey	1955	3	65.52
P.I. 206453	sativa	diploid	Turkey	1955	4	61.55
P.I. 204886	falcata	tetraploid	Turkey	1959	3	60.72
P.I. 179370	sativa	diploid	Turkey	----	4	54.55
P.I. 172984	sativa	diploid	Turkey	----	4	54.55
F.C. 24280	falcata	diploid	So. Armodt	----	4	48.96
P.I. 212798	sativa	diploid	Iran	----	4	48.85
P.I. 172989	sativa	diploid	Turkey	----	4	41.30
LSD 5%						21.31
LSD 1%						28.46

1/ Diameter of seed as described in Table 4.

2/ Average of four replications.

3/ Date of harvest unknown. Seed was received February, 1958.

SUMMARY

The adopted method of testing for *Pythium* resistance in alfalfa involved sprinkling metal flats of steam sterilized "fine" mason's sand with a 500 ml. *Pythium* suspension 1 day prior to planting. The suspension was prepared by blending 3-day-old *Pythium* cultures on 2 percent PDA from two Petri dishes with 200 ml. of distilled water for 20 seconds and diluting to 500 ml. with distilled water.

Increased seed size was found directly associated with improved seedling stands in *Pythium* infested sand. More uniform seedling stands were attained with seeding rates of 1, 2, or 5 than with 10 seeds per inch as the pathogen tended to spread from plant to plant at the higher seeding rates.

Differences in *Pythium* resistance were found among seed lots of certified Buffalo alfalfa produced in seven states during 1960. Near significant differences were found among seed lots of certified Buffalo produced in a localized area of Utah in 1960.

First cycle selections from six of eight alfalfa varieties were significantly more *Pythium* tolerant than their parent varieties. However, second cycle selections in a study involving only Cody were not significantly more resistant than first cycle selections.

Interaction between alfalfa varieties or selections and three *Pythium* species was not significant. However, highly significant differences existed both among resistance levels of the alfalfa entries and in the ability of the *Pythium* species to in-

cite damping-off.

The manner of expression and amount of *Pythium* damage was strongly associated with temperature. Pre-emergence damping-off was greater at 60° than at 82° F., while postemergence damping-off was more severe at 82° F. Total seedling survival was lower at 60° F. A significant temperature x variety interaction was found; however, the increase in plant survival by selection appeared to be independent of temperature.

Highly significant differences were found in levels of *Pythium* resistance expressed by 21 alfalfa varieties although none approached immunity. Fifty *Medicago sativa* and *M. falcata* introductions including diploids and tetraploids exhibited wide levels of *Pythium* resistance. However, none were significantly more resistant than previously tested varieties with which they were compared.

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PYTHIUM RESISTANCE IN ALFALFA

by

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AN ABSTRACT OF A THESIS

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The *Pythium* species, world-wide in distribution, are one of the more common incitants of damping-off and contribute regularly to seedling losses in alfalfa. Information regarding *Pythium* resistance in alfalfa is meager. Therefore, a study was initiated to determine methods of testing for *Pythium* resistance in alfalfa, factors affecting resistance, sources of resistance, the possibilities of increasing resistance by selection, and to study the relative reaction of alfalfa varieties and selections to different *Pythium* species.

The method adopted for testing involved sprinkling metal flats of steam sterilized, "fine" mason's sand with a 500 ml. *Pythium* suspension 1 day prior to planting. The suspension was prepared by blending 3-day-old *Pythium* cultures on 2 percent potato-dextrose agar from two Petri plates with 200 ml. of distilled water for 20 seconds and diluting to 500 ml. with distilled water.

Seed size contributed to seedling survival. Alfalfa seeds passing through a 1.27-mm. screen were significantly more susceptible to damping-off than larger seeds.

More uniform results were obtained with seeding rates of 1, 2, or 5 than with 10 seeds per inch, as the pathogen tended to spread from plant to plant at the heavier seeding rate.

Seedlings from certified Buffalo alfalfa seed lots produced in seven states during 1960 differed significantly in their ability to survive *Pythium* infestation.

Seedling survival of 16 alfalfa varieties planted in sand

infested with *P. debaryanum* differed significantly although no varieties approached immunity. Five-plant, intravarietal intercrosses were made from surviving plants of eight varieties. Equal numbers of seeds from each plant included in an intercross were bulked and compared for resistance with the parent seed lot. Six intercrosses were significantly more resistant than the parent variety.

In a study involving only Cody alfalfa, three of seven first cycle selections were significantly more tolerant to *Pythium* than Cody. However, *Pythium* tolerance expressed by second cycle selections did not significantly exceed that of first cycle selections.

Wide differences in seedling survival among 50 alfalfa Plant Introductions from several countries were apparent. However, none were significantly more resistant to *Pythium* attack than previously tested varieties with which they were compared.

Eight varieties and five selections from Cody were tested with *P. debaryanum* Hesse, *P. irregulare* Buis., and *P. ultimum* Trow to determine the reaction of alfalfa varieties and selections to different *Pythium* species. A difference in virulence was apparent among the *Pythium* species but no interaction was detected between the alfalfa varieties or selections and the *Pythium* species used.

The manner of expression and amount of *Pythium* damage was strongly associated with temperature. Pre-emergence damping-off was greater at 60° than at 82° F., while postemergence damping-off

was more severe at 82° F. Total seedling survival was lower at 60° F. A significant temperature x variety interaction was found; however, the increase in plant survival by selection appeared to be independent of temperature.